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Blood Lead Levels and Risk of Atherosclerosis in the Carotid Artery: Results from a Swedish Cohort

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Background: Lead exposure has been associated with increased incidence of adverse clinical cardiovascular outcomes. Atherosclerosis has been suggested as one of the underlying mechanisms, and findings from experimental studies support this, but human data are scarce.

Objectives: Our objective was to determine the association between environmental lead exposure based on blood lead (B-Pb) concentrations and the prevalence of atherosclerotic plaque in the carotid artery.

Methods: We used cross-sectional data from the Malmö Diet and Cancer Study cardiovascular cohort (MDCS-CC; recruitment in 1991–1994) covering 4,172 middle-aged men and women. B-Pb at baseline, measured by inductively coupled plasma mass spectrometry, was used as the exposure biomarker. The presence of atherosclerotic plaque in the carotid artery was determined by B-mode ultrasonography. We used logistic regression to estimate odds ratios (ORs) for prevalence of plaque in the carotid artery according to B-Pb quartiles.

Results: The median B-Pb was 25 μg/L (range: 1.5–258), and 36% of the cohort had any atherosclerotic plaque. After controlling for confounders and known cardiovascular risk factors, the OR for prevalence of plaque in the highest quartile (Q4) of B-Pb compared with the lowest quartile (Q1) was 1.35 (95% CI: 1.09, 1.66) in the total group, 1.58 (95% CI: 1.20, 2.08) among women, and 1.18 (95% CI: 0.83, 1.69) among men. Among women, associations were limited to those who were postmenopausal [OR for Q4 vs. Q1 = 1.72 (95% CI: 1.26, 2.34) vs. OR = 0.96 (95% CI: 0.49, 1.89 in premenopausal women)]. Associations were weak and nonsignificant in never-smokers [OR for Q4 vs. Q1 = 1.14 (95% CI: 0.81, 1.61)].

Discussion: Our study shows an association between B-Pb concentrations and occurrence of atherosclerotic plaque in the carotid artery, adding evidence for an underlying pro-atherogenic role of lead in cardiovascular disease. Associations appeared to be limited to postmenopausal (vs. premenopausal) women. https://doi.org/10.1289/EHP5057

Introduction

Environmental exposure to the toxic metal lead (Pb) remains a public health problem. Despite a considerable decrease in lead exposure in the last two decades, there are still reports of health effects in children and adults even at low levels of exposure (NTP 2012; Skerfving and Bergdahl 2015). Exposure to lead occurs mainly via contaminated food, drinking water, and dust but also through smoking and industrial and combustion emissions (EFSA 2010; Skerfving and Bergdahl 2015). Most of the absorbed lead is stored for decades in bones (Barry 1975; Silbergeld et al. 1993; Skerfving and Bergdahl 2015), and so bone lead mirrors both long-term exposure and body burden. Lead in blood (B-Pb) reflects more recent exposure, as well as the whole body burden, and is the most commonly used biomarker of lead exposure (Barbosa et al. 2005; Skerfving and Bergdahl 2015).

Cardiovascular diseases continue to be the leading cause of death and the largest contributor to premature mortality worldwide (GBD 2016 Causes of Death Collaborators 2017). In particular, ischemic heart disease and cerebrovascular disease account for nearly 10 million and 5 million deaths globally every year, respectively (GBD 2016 Causes of Death Collaborators 2017). Lead exposure is associated with an increased incidence of cardiovascular disease (EFSA 2010; Navas-Acien et al. 2007; NTP 2012; Skerfving and Bergdahl 2015) and has been estimated to account for at least 240,000 ischemic heart disease deaths per year globally (GBD 2015 Causes of Death Collaborators 2016). Evidence on lead toxicity in the cardiovascular system is particularly extensive with regard to the link between lead exposure and increased blood pressure and hypertension even at concentrations <100 μg/L (Navas-Acien et al. 2007; NTP 2012; U.S. EPA 2006). However, the impact of lead on the cardiovascular system is not restricted to effects on blood pressure. There is an increasing body of evidence suggesting an association between low-level lead exposure (concentrations <50 μg/L) and clinical cardiovascular outcomes such as peripheral arterial disease, cardiovascular mortality, and coronary heart disease (Lanphear et al. 2018; Navas-Acien et al. 2007; NTP 2012; Skerfving and Bergdahl 2015).

Atherosclerosis has been suggested to be one of the underlying mechanisms for the cardiovascular toxicity of lead (Lanphear et al. 2018; Vaziri 2008), and there are findings from experimental studies to support this (Fujiwara et al. 1995; Zeller et al. 2010). Evidence from epidemiological studies showing an association between low-level lead exposure and clinical cardiovascular outcomes also suggests atherosclerotic changes (Lanphear et al. 2018; Navas-Acien et al. 2007; NTP 2012; Skerfving and Bergdahl 2015). However, we found no epidemiological studies showing an association between environmental lead exposure and the incidence or presence of atherosclerotic plaques. In the present study, we aimed to assess the association between B-Pb and prevalence of atherosclerotic plaque in the carotid artery in a Swedish population-based cohort.

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Methods

Study Design and Population

This study was based on cross-sectional baseline data from the cardiovascular cohort of the Malmö Diet and Cancer Study (MDCS-CC) (Rosvall et al. 2000, 2015). The MDCS-CC is a random sample of the larger population-based Malmö Diet and Cancer Study (MDCS) (Berglund et al. 1993; Manjer et al. 2001), and comprises 6,103 individuals 46–67 years of age living in Malmö, Sweden, between 1991 and 1994 (Rosvall et al. 2000) who were selected for a substudy on the epidemiology of carotid artery disease. The present study included all individuals for whom data were available on ultrasound-assessed plaque status in the carotid artery and B-Pb ($n = 4{,}172$; Figure 1).

The study was carried out in accordance with the Declaration of Helsinki. MDCS-CC was approved by the Ethics Committee of Lund University, Lund, Sweden (MDCLU51–90). All participants provided informed consent.

Analyses of Lead Concentrations in Blood

Venous blood samples were collected at baseline after overnight fast. Plasma and erythrocytes were separated, and lead was measured in erythrocytes using inductively coupled plasma mass spectrometry with an octopole reaction system in helium mode (Agilent 7700x ICP-MS; Agilent Technologies) at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden (Gambelunghe et al. 2016; Harari et al. 2018). Lead in whole blood was then calculated by multiplying the metal concentrations in erythrocytes by the hematocrit. None of the samples were below the limit of detection, which was 0.16 μg/L. External quality control samples with low lead levels (Seronorm Trace Elements Whole Blood L-1, Lot no. 1103128; Sero AS) were included in all analytical rounds and showed satisfactory results: mean ± standard deviation (SD) 10.3 ± 0.5 μg/L vs. recommended limits of 6–14 μg/L. The imprecision (coefficient of variation) was 5.1%, calculated from duplicate samples.

Measurement of Atherosclerotic Plaque in the Carotid Artery

B-mode ultrasonography (Acuson 128 CT System; Siemens) of the right carotid artery was performed to assess plaque occurrence according to a standardized protocol, by trained, certified sonographers (Rosvall et al. 2000). In brief, the bifurcation area of the right common carotid artery was scanned within a predefined window comprising 3 cm of the distal common carotid artery, the bifurcation, and 1 cm of the internal and external carotid artery, respectively, for the occurrence of plaques. Plaque was defined as a focal thickening of the intima–media complex >1.2 mm with an area ≥10 mm^2. Methods of quality control were used as described previously (Rosvall et al. 2000). The reliability of the measurements was assured by regularly (about every 3 weeks) performing intra- and interobserver variability analyses during the ultrasound investigation procedure (Rosvall et al. 2000). Previous intra- and interobserver analyses have been reported elsewhere (Persson et al. 1992). Intra-observer variability for three ultrasound operators were 10%, 6%, and 10%, respectively for intima–media thickness of the common carotid artery (IMT CCA). Interobserver variability of the IMT CCA for three pairs of observers was $r = 0.66$, $r = 0.94$, and $r = 0.87$, respectively.

Cardiovascular Risk Factors

All participants filled in a self-administered questionnaire at baseline concerning lifestyle, health, occupation, and medication (Rosvall et al. 2000). Participants were categorized into never or ever (including both current and former) smokers. Menopausal status of women was assessed using both questionnaire data and medical records. A woman was classified as postmenopausal if *a*) she had undergone bilateral oophorectomy (medical records, $n = 45$), or *b*) the above criteria was missing and she confirmed that her menstruation had ceased ≥2 y prior to baseline (questionnaire, $n = 1{,}797$), or *c*) the above criteria were absent and she was ≥55 years of age ($n = 20$). Pack-years of smoking were calculated from years of smoking and the amount of tobacco smoked daily in the

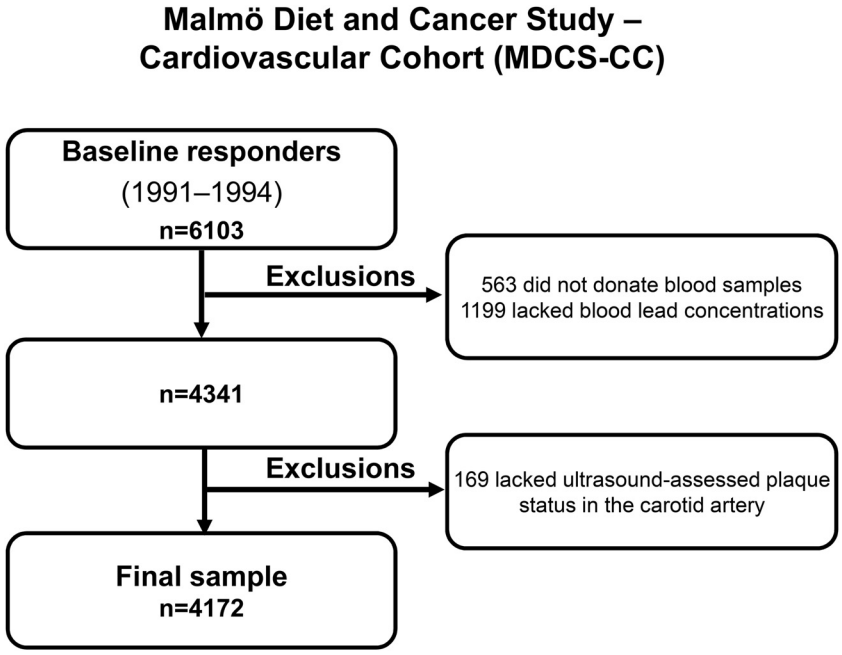

Figure 1. Cohort diagram illustrating the study population. The reason for missing blood lead concentrations in 1,199 participants was lack of blood sample available in the biobank for lead analysis. The lack of ultrasound-assessed plaque status in the carotid artery in 169 participants was due to technical difficulties.

subgroup with such data available ($n = 3{,}422$ of which 1,744 were former and current smokers) (Fagerberg et al. 2015). Daily alcohol intake was calculated in grams per day. Blood pressure, height, weight, and waist circumference were measured, and body mass index (BMI) was calculated. Hypertension was defined as an average systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg and/or current use of antihypertensive medication prescribed by a physician. Participants were classified as having diabetes mellitus if they reported the diagnosis in the questionnaire, used anti-diabetic medication, or had a fasting venous whole blood glucose level of ≥ 6.1 mmol/L. Educational level was classified as low if participants had completed <9 y of education (i.e., had not completed secondary education). Low physical activity was defined as the lowest quartile of a summary score of physical activity based on a composite measure of 18 different leisure-time activities during the preceding year, involving information on activity intensity and time spent on the activity (Li et al. 2009). Information on occupation was obtained from the baseline questionnaire and the most recent occupation was considered at the time of the baseline examination. The occupations were coded according to the job classification scheme Population and Housing Census from 1980 (FoB-80) and thereafter classified into blue-collar, white-collar, or self-employed workers (Rosvall et al. 2000). Overnight fasting blood samples were drawn for determination of hemoglobin A1c (HbA1c), whole blood glucose, high sensitivity C-reactive protein (hsCRP), high density lipoprotein (HDL), total cholesterol, low density lipoprotein (LDL), and triglycerides (Fagerberg et al. 2015).

Statistical Analyses

Statistical analyses were performed with Stata (release 14.1; Stata Corporation). A two-tailed $p < 0.05$ was considered statistically significant. Initial bivariate associations between B-Pb and cardiovascular risk factors and potential confounders were assessed with Spearman's rank correlation (r_s) for continuous variables and the Mann-Whitney test for categorical variables. Bivariate associations between plaque prevalence and cardiovascular risk factors and potential confounders were assessed using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables.

Descriptive statistics were calculated by quartiles of B-Pb and presented as median (range) for nonnormal distributed variables, mean ± SD for normally distributed variables, and proportions for categorical variables. Differences across quartiles of B-Pb were tested using the Kruskal-Wallis H rank test for continuous variables and the chi-square test for categorical variables.

Associations between B-Pb and plaque prevalence were analyzed using multivariable-adjusted logistic regression analyses with B-Pb in quartiles. As well as a crude model (Model 1), we used an adjusted model (Model 2) that included variables associated with both B-Pb and occurrence of plaque: age (years), sex (female vs. male), smoking (never, former, or current), hypertension (yes vs. no), and waist circumference (in centimeters). A final model (Model 3) was additionally adjusted for other confounders and known cardiovascular risk factors as well as factors that may be in the pathway of B-Pb and atherosclerosis: HbA1c (as a proxy for degree of glucose intolerance), total cholesterol/HDL ratio, statin treatment (yes vs. no), hsCRP, low education level (≤ vs. >9 y), and physical activity (low vs. high). We performed complete case analyses for each model.

Initially, we examined the associations in all individuals. Subsequently, we used multiplicative interaction terms using B-Pb in quartiles and the stratifying factor (i.e., smoking status (never/ever smokers), hypertension (no/yes), menopausal status (pre-/postmenopausal), and estimated glomerular filtration rate (eGFR) categories (>90, 60–90, and <60 mL/min per 1.73 m^2) and report the $p_{interaction}$ term of Q4 vs. Q1 and the highest category of the stratification factor. We then stratified the models by these factors to assess potential effect modification. Linear trend p-values were derived by modeling the median value of each B-Pb quartile as a continuous variable in the models.

We evaluated nonlinearity both by introducing a quadratic term for B-Pb levels in Model 3 as well as by estimating the smoothed exposure–response relationship between B-Pb levels (with and without log-transformation) and plaque (adjusted for the same covariates as Model 3), in all individuals as well as separately in men and women, using generalized additive models with the procedure gam in the mgcv package in R (version 3.3.2; R Development Core Team), where the smooth term was represented using a penalized regression spline (with 1.285 equivalent degrees of freedom). Figures show the log(odds) relabeled to odds ratios (ORs).

We performed several sensitivity analyses. We adjusted the models for pack-years instead of smoking status. We categorized pack-years in five categories: one for never-smokers (Category 1: 0 pack-years) and four based on quartiles among ever-smokers (Category 2: >0 but <7 pack-years, Category 3: ≥7 but <16.3 pack-years, Category 4: ≥16.3 but <30 pack-years, and Category 5: ≥30 pack-years). We tested the influence of kidney function in the associations using serum creatinine eGFR (Harari et al. 2018). We included blood cadmium in adjusted models as an additional potential confounder (Bergström et al. 2015; Fagerberg et al. 2015). We adjusted Model 3 additionally for an alternative indicator of socioeconomic status, namely occupation (blue-collar, white-collar, and self-employed workers). We also adjusted Model 3 for diabetes (yes/no) instead of HbA1c as well as for BMI instead of waist circumference. In order to assess whether the associations seen among postmenopausal women were driven and explained by age, we performed stratified analyses by age (< or ≥55 y). We also performed the regressions again using B-Pb as a continuous variable instead of in quartiles and, finally, we excluded individuals with B-Pb >100 μg/L in these analyses to assess whether the associations between B-Pb (as continuous variable) and atherosclerosis were driven by individuals with very high lead levels.

Results

Cohort Characteristics

Characteristics of all MDCS-CC participants by quartiles of B-Pb are presented in Table 1. The median B-Pb was 25 μg/L (range: 1.5–258; see Figure S1A). Alcohol intake, blood cadmium and waist circumference as well as the proportion of men, current smokers, self-employed workers, and individuals with hypertension were higher in the highest quartile (Q4) of B-Pb as compared with the three lowest quartiles (Q1–Q3). On the contrary, the proportion of blue-collar workers decreased with increasing quartiles of B-Pb. Both systolic and diastolic blood pressure increased with increasing quartiles of B-Pb.

The study participants were 60% women and had a mean age of 57 y (range: 46–67), a mean waist circumference of 83 cm (range: 54–152), and a mean BMI of 26 kg/m^2 (range: 16–51). About 63% had hypertension, whereas 7.8% had diabetes and 1.4% received statin treatment.

The number of observations with missing covariate data was as follows: postmenopausal status, $n = 7$; low education, $n = 10$; low physical activity, $n = 101$; BMI, $n = 3$; waist, $n = 3$; alcohol consumption, $n = 19$; eGFR, $n = 65$; and occupation, $n = 24$. Ninety one percent (3,807 of 4,172) of the individuals had complete data for all covariates included in the fully adjusted model (Model 3).

Table 1. Characteristics of the study participants by quartiles (Q1–Q4) of blood lead in the Malmö Diet and Cancer Study–Cardiovascular Cohort (MDCS-CC).

Characteristics[a]	Overall	Quartiles of blood lead				
		Q1	Q2	Q3	Q4	
MDCS-CC	$n = 4{,}172$	$n = 1{,}040$	$n = 1{,}042$	$n = 1{,}044$	$n = 1{,}046$	p-Value[b]
Plaque occurrence in carotid artery	1,482 (36)	317 (30.5)	370 (35.5)	368 (35.3)	427 (40.8)	<0.001
Blood lead (μg/L)	25 (1.5–258)	15 (1.5–18)	21 (18–25)	28 (25–33)	42 (33–258)	<0.001
Age (y)	57 ± 5.9	57 ± 6.2	58 ± 5.8	57 ± 5.8	57 ± 5.7	0.30
Sex						<0.001
Male	1,651 (40)	227 (22)	348 (33)	472 (45)	604 (58)	
Female	2,521 (60)	813 (78)	694 (67)	572 (55)	442 (42)	
Menopausal status						0.003
Premenopausal	652 (26)	246 (30)	168 (24)	142 (25)	96 (22)	
Postmenopausal	1,862 (74)	564 (70)	526 (76)	427 (75)	345 (78)	
Blood cadmium (μg/L)	0.26 (0.034–5.1)	0.22 (0.04–4.2)	0.24 (0.06–4.3)	0.28 (0.04–4.8)	0.33 (0.03–5.1)	<0.001
Low education level						0.056
Yes	1,929 (46)	499 (48)	507 (49)	466 (45)	457 (44)	
No	2,233 (54)	541 (52)	530 (51)	577 (55)	585 (56)	
Low physical activity						0.54
Yes	953 (23)	231 (23)	244 (24)	227 (22)	251 (25)	
No	3,118 (77)	782 (77)	775 (76)	796 (78)	765 (75)	
Body mass index (kg/m^2)	26 ± 3.8	26 ± 4.3	26 ± 3.9	25 ± 3.5	25 ± 3.5	0.96
Waist circumference (cm)	83 ± 13	81 ± 13	82 ± 13	84 ± 12	85 ± 12	<0.001
Smoking status						
Never-smokers	1,678 (40)	532 (51)	479 (46)	364 (35)	303 (29)	<0.001
Former smokers	1,404 (34)	331 (32)	314 (30)	401 (38)	358 (34)	<0.001
Current smokers	1,090 (26)	177 (17)	249 (24)	279 (27)	385 (37)	<0.001
Pack-years[c]	17 (0.06–158)	14.4 (0.1–140)	15 (0.2–93)	16 (0.1–145)	20 (0.06–158)	<0.001
Alcohol consumption (g/d)	6.8 (0–105)	3.0 (0–44)	5.3 (0–80)	8.3 (0–86)	13 (0–105)	<0.001
Hypertension						<0.001
Yes	2,614 (63)	645 (62)	619 (59)	639 (61)	711 (68)	
No	1,558 (37)	395 (38)	423 (41)	405 (39)	335 (32)	
Systolic blood pressure (mmHg)	140 ± 19	140 ± 18	139 ± 18	141 ± 19	142 ± 19	0.0036
Diastolic blood pressure (mmHg)	87 ± 9.2	86 ± 8.7	86 ± 9.2	87 ± 9.2	88 ± 9.7	<0.001
Diabetes mellitus						0.31
Yes	310 (7.4)	91 (8.8)	71 (6.8)	73 (7.0)	75 (7.2)	
No	3,862 (92.6)	949 (91.2)	971 (93.2)	971 (93.0)	971 (92.8)	
Statin treatment						0.76
Yes	58 (1.4)	13 (1.3)	16 (1.5)	17 (1.6)	12 (1.2)	
No	4,114 (98.6)	1,027 (98.7)	1,026 (98.5)	1,027 (98.4)	1,034 (98.8)	
Total cholesterol/HDL ratio	4.7 ± 1.5	4.7 ± 1.5	4.7 ± 1.4	4.8 ± 1.5	4.8 ± 1.5	0.11
HbA1c (%)	4.8 (3.3–12)	4.7 (3.5–12)	4.8 (3.3–11)	4.7 (3.3–12)	4.8 (3.5–12)	0.021
hsCRP (mg/mL)	1.3 (0.09–60)	1.3 (0.09–60)	1.3 (0.09–56)	1.4 (0.09–53)	1.4 (0.09–58)	0.054
$eGFR_{cr}$ (mL/min per 1.73 m^2)	76 ± 14	76 ± 14	75 ± 14	76 ± 13	76 ± 14	0.21
Occupation						
Blue-collar workers	1,571 (38)	439 (43)	413 (40)	369 (36)	350 (34)	<0.001
White-collar workers	2,157 (52)	524 (51)	541 (52)	556 (54)	536 (51)	0.64
Self-employed workers	420 (10)	68 (7)	84 (8)	113 (11)	155 (15)	<0.001
History of stroke						0.27
Yes	26 (0.6)	10 (1.0)	7 (0.7)	3 (0.3)	6 (0.6)	
No	4,146 (99.4)	1,030 (99.0)	1,035 (99.3)	1,041 (99.7)	1,040 (99.4)	
History of myocardial infarction						0.57
Yes	58 (1.4)	13 (1.3)	11 (1.1)	18 (1.7)	16 (1.5)	
No	4,114 (98.6)	1,027 (98.7)	1,031 (98.9)	1,026 (98.3)	1,030 (98.5)	

Note: Median (range), mean ± SD, or n (%) are presented.
[a]Missing data: postmenopausal status $n = 7$, low education level $n = 10$, low physical activity $n = 101$, BMI $n = 3$, waist $n = 3$, pack-years $n = 750$, alcohol consumption $n = 19$, eGFR $n = 65$, and occupation $n = 24$. The missing data were relatively evenly distributed by quartiles of blood lead.
[b]p-Value obtained using Kruskal-Wallis H test for continuous variables and chi-square test for categorical variables.
[c]Only former and current smokers ($n = 1{,}744$).

Men differed from women in several ways (see Table S1). In particular, men showed higher B-Pb (median 29 μg/L in men vs. 22 in women; see Figure S1B), higher prevalence of plaque (39% vs. 33%), and higher prevalence of cardiovascular risk factors. Seventy-four percent of the women were postmenopausal. In comparison with premenopausal women, postmenopausal women had slightly higher B-Pb, and they were on average 9 y older (median B-Pb: 21 μg/L vs. 23 μg/L; mean age: 51 y vs. 60 y in pre- and postmenopausal women, respectively; $p < 0.001$ for both).

Individuals with atherosclerotic plaque in the carotid artery had statistically significantly higher B-Pb levels as well as a higher prevalence of cardiovascular risk factors compared with those without carotid plaque (see Table S2). Specifically, individuals with carotid plaque were older, had a larger waist circumference as well as higher blood pressure and HbA1c levels, were mostly current smokers, and had a higher prevalence of hypertension, diabetes, and statin treatment.

B-Pb was correlated with blood cadmium levels ($r_s = 0.22$, $p < 0.001$), waist circumference ($r_s = 0.16$, $p < 0.001$), pack-years ($r_s = 0.24$, $p < 0.001$), alcohol intake ($r_s = 0.38$, $p < 0.001$), systolic ($r_s = 0.05$, $p = 0.007$) and diastolic blood pressure ($r_s = 0.10$, $p < 0.001$), and total cholesterol/HDL ratio ($r_s = 0.05$, $p = 0.005$).

Association between Blood Lead and Plaque

The prevalence of plaque in the carotid artery was 36% overall, and 10% higher in Q4 than in Q1 ($p < 0.001$) (Table 1). Table 2 shows results from the unadjusted logistic regression model (Model 1); the model adjusted for smoking, waist circumference, hypertension, sex, and age (Model 2); and the model additionally adjusted for total cholesterol/HDL ratio, hsCRP, statin treatment, HbA1c, education level, and physical activity (Model 3). Model 1 showed an increased risk for occurrence of carotid plaque in Q4 as compared with Q1 for all participants as well as for women when stratifying by sex. After adjustment, ORs decreased but remained statistically significant. Overall, in Model 3 Q4 showed a 35% increased OR [95% confidence interval (CI): 1.09, 1.66] for plaque in the carotid artery in comparison with Q1 ($p_{trend} = 0.011$). Among women, those in Q4 had a 58% increased risk (95% CI: 1.20, 2.08) in comparison with Q1 ($p_{trend} = 0.002$; Table 2), whereas there was no such trend in men [OR for Q4 vs. Q1 = 1.18 (95% CI: 0.83, 1.69), $p_{trend} = 0.76$]. The $p_{interaction}$ between sex and the OR for Q4 was 0.21 (see footnote to Table 2). Adjusting Model 1 only for age and sex modified the estimated only slightly (see Table S3).

When the statistical analyses were stratified by smoking status, ORs became weaker and nonsignificant for never smokers [OR for Q4 vs. Q1 = 1.14 (95% CI: 0.81, 1.61)], whereas they were stronger and significant for ever smokers [OR for Q4 vs. Q1 = 1.61 (95% CI: 1.23, 2.12), $p_{trend} = 0.002$, $p_{interaction} = 0.10$; Table 3]. When stratifying by menopausal status, postmenopausal women ($n = 1{,}695$ with complete data on covariates) showed an increased risk [OR for Q4 vs. Q1 = 1.72 (95% CI: 1.26, 2.34), $p_{trend} = 0.001$; Figure 2 and Table 3]. The $p_{interaction}$ between menopausal status and quartiles of B-Pb (Q4 compared with Q1) was 0.11. We found no associations between B-Pb and risk of atherosclerosis in premenopausal women [$n = 603$; OR for Q4 vs. Q1 = 0.96 (95% CI: 0.49, 1.89)]. Stratification of Model 3 by hypertension showed similar risks in individuals with and without hypertension ($p_{interaction} = 0.82$; Table 3).

Stratifying Model 3 by eGFR showed strongest estimates for individuals with decreased kidney function. In individuals with eGFR <60 mL/min per 1.73 m^2, the OR for Q4 vs. Q1 = 2.16 [(95% CI: 1.10, 4.24), $n = 414$, $p_{trend} = 0.06$]. In individuals with eGFR 60–90 mL/min per 1.73 m^2 the OR for Q4 vs. Q1 = 1.25 [(95% CI: 0.98, 1.59), $n = 2{,}761$, $p_{trend} = 0.07$], and in individuals with eGFR >90 mL/min per 1.73 m^2, the OR for Q4 vs. Q1 = 1.27 [(95% CI: 0.71, 2.28), $n = 602$, $p_{trend} = 0.72$; Table 3 and Figure S2].

Introducing a quadratic term of the B-Pb concentrations in Model 3 to assess nonlinearity did not show evidence of nonlinearity ($p = 0.16$). This is consistent with Figure 3, showing a linear association in the smoothed exposure–response relation for carotid plaque based on B-Pb as a continuous variable (Figure 3 for B-Pb < 150 µg/L; see Figure S3 for all individuals). The smoothed exposure–response relation in men and women separately are consistent with the findings in Table 2 (see Figure S4A for men and S4B for women). Log-transformation of B-Pb showed no influence of extreme values on the association between B-Pb and atherosclerosis (see Figure S5).

Sensitivity Analyses

Adjusting the statistical models for pack-years in those individuals with data available and using five categories as described above instead of smoking categories showed essentially the same results concerning associations between B-Pb and atherosclerosis in all individuals as well as in men and women separately (see Table S4). We also tested the influence of kidney function by adjusting the models for creatinine-based eGFR, but the estimates remained unchanged (see Table S5). We also examined whether the estimates from Model 3 changed by introducing blood cadmium in the model, but ORs remained essentially unchanged (see Table S6). We adjusted Model 3 also for occupation (blue-collar, white-collar, and self-employed workers) and for diabetes (instead of HbA1c), but these additional adjustments did not change the

Table 2. Logistic regression models of associations of blood lead (in quartiles) with occurrence of plaque in the carotid artery in the Malmö Diet and Cancer Study–Cardiovascular Cohort (MDCS-CC), in all individuals and stratified by sex.

	Model 1[a]			Model 2[b]			Model 3[c]		
Quartiles of blood lead [range (µg/L)]	With plaque [*n* (%)]	Without plaque [*n* (%)]	OR (95% CI)	With plaque [*n* (%)]	Without plaque [*n* (%)]	OR (95% CI)	With plaque [*n* (%)]	Without plaque [*n* (%)]	OR (95% CI)
All[d]									
Q1 (1.5–18)	317 (30)	723 (70)	Ref	317 (31)	722 (69)	Ref	295 (31)	669 (69)	Ref
Q2 (18–25)	370 (36)	672 (64)	1.26 (1.05, 1.51)	370 (36)	672 (64)	1.18 (0.97, 1.43)	338 (36)	611 (64)	1.19 (0.97, 1.46)
Q3 (25–33)	368 (35)	676 (65)	1.24 (1.03, 1.49)	367 (35)	676 (65)	1.11 (0.91, 1.35)	333 (35)	618 (65)	1.13 (0.92, 1.39)
Q4 (33–258)	427 (41)	619 (59)	1.57 (1.31, 1.88)	426 (41)	619 (59)	1.31 (1.07, 1.59)	379 (40)	564 (60)	1.35 (1.09, 1.66)
p_{trend}[e]			<0.001			0.018			0.011
Women[d]									
Q1 (1.5–18)	234 (29)	579 (71)	Ref	234 (29)	578 (71)	Ref	217 (29)	535 (71)	Ref
Q2 (18–25)	227 (33)	467 (67)	1.20 (0.97, 1.50)	227 (33)	467 (67)	1.14 (0.91, 1.44)	206 (32)	429 (68)	1.13 (0.89, 1.44)
Q3 (25–33)	190 (33)	382 (67)	1.23 (0.98, 1.55)	190 (33)	382 (67)	1.11 (0.87, 1.41)	171 (33)	353 (67)	1.13 (0.87, 1.45)
Q4 (33–258)	180 (41)	262 (59)	1.70 (1.33, 2.17)	180 (41)	262 (59)	1.50 (1.16, 1.94)	158 (40)	235 (60)	1.58 (1.20, 2.08)
p_{trend}[e]			<0.001			0.004			0.002
Men[d]									
Q1 (2.7–18)	83 (37)	144 (63)	Ref	83 (37)	144 (63)	Ref	78 (37)	134 (63)	Ref
Q2 (18–25)	143 (41)	205 (59)	1.21 (0.86, 1.71)	143 (41)	205 (59)	1.22 (0.85, 1.76)	132 (42)	182 (58)	1.32 (0.90, 1.94)
Q3 (25–33)	178 (38)	294 (62)	1.05 (0.76, 1.46)	177 (38)	294 (62)	1.06 (0.75, 1.50)	162 (38)	265 (62)	1.11 (0.77, 1.60)
Q4 (33–173)	247 (41)	357 (59)	1.20 (0.88, 1.64)	246 (41)	357 (59)	1.13 (0.81, 1.58)	221 (40)	329 (60)	1.18 (0.83, 1.69)
p_{trend}[e]			0.39			0.82			0.76

Note: $p_{Interaction}$ term of Q4 × sex in Model 3 with all individuals = 0.21. CI, confidence interval; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; OR, odds ratio; Q, quartile; Ref, reference.

[a]Model 1: Unadjusted.

[b]Model 2: Adjusted for smoking, waist circumference, hypertension, sex, and age.

[c]Model 3: Additionally adjusted for total cholesterol/HDL ratio, C-reactive protein, statin treatment, HbA1c, education level, and physical activity.

[d]Model 1: $n = 4{,}172$ (all), $n = 2{,}521$ (women), $n = 1{,}651$ (men); Model 2: $n = 4{,}169$ (all), $n = 2{,}520$ (women), $n = 1{,}649$ (men); Model 3: $n = 3{,}807$ (all), $n = 2{,}304$ (women), $n = 1{,}503$ (men).

[e]Linear trend *p*-values were derived by modeling the median value of each blood lead quartile as a continuous variable in the models.

Table 3. Logistic regression models (Model 3 from Table 2) of associations of blood lead (in quartiles) with occurrence of carotid plaque in the Malmö Diet and Cancer Study–Cardiovascular Cohort (MDCS-CC) stratified by characteristics (smoking status, hypertension, menopausal status, and eGFR).

	Quartiles of blood lead				
Characteristics	Q1	Q2	Q3	Q4	p_{Trend}[a]
Blood lead [range (μg/L)]	1.5–18	18–25	25–33	33–258	
Smoking status[b]					
Never smokers ($n = 1{,}561$)					
n with plaque	144	139	106	87	
n without plaque	355	310	228	192	
OR (95% CI)	1.0	1.06 (0.80, 1.42)	1.14 (0.83, 1.57)	1.14 (0.81, 1.61)	0.40
Ever smokers ($n = 2{,}246$)					
n with plaque	151	199	227	292	
n without plaque	314	301	390	372	
OR (95% CI)	1.0	1.39 (1.05, 1.84)	1.20 (0.91, 1.58)	1.61 (1.23, 2.12)	0.002
Hypertension[c]					
No ($n = 1{,}444$)					
n with plaque	78	105	92	94	
n without plaque	290	282	285	218	
OR (95% CI)	1.0	1.25 (0.88, 1.79)	1.12 (0.77, 1.61)	1.48 (1.01, 2.16)	0.071
Yes ($n = 2{,}363$)					
n with plaque	217	233	241	285	
n without plaque	379	329	333	346	
OR (95% CI)	1.0	1.16 (0.90, 1.48)	1.17 (0.91, 1.50)	1.30 (1.01, 1.68)	0.053
Menopausal status[d]					
Premenopausal women ($n = 603$)					
n with plaque	38	31	21	17	
n without plaque	188	125	113	70	
OR (95% CI)	1.0	1.08 (0.63, 1.88)	0.77 (0.42, 1.42)	0.96 (0.49, 1.89)	0.69
Postmenopausal women ($n = 1{,}695$)					
n with plaque	178	175	149	141	
n without plaque	345	304	239	164	
OR (95% CI)	1.0	1.14 (0.87, 1.49)	1.19 (0.90, 1.59)	1.72 (1.26, 2.34)	0.001
eGFR categories[e]					
eGFR >90 mL/min per 1.73 m^2 ($n = 602$)					
n with plaque	34	46	38	54	
n without plaque	122	95	99	114	
OR (95% CI)	1.0	1.51 (0.85, 2.69)	1.15 (0.63, 2.09)	1.27 (0.71, 2.28)	0.72
eGFR 60–90 mL/min per 1.73 m^2 ($n = 2{,}761$)					
n with plaque	230	229	252	285	
n without plaque	476	432	454	403	
OR (95% CI)	1.0	1.06 (0.83, 1.34)	1.05 (0.83, 1.33)	1.25 (0.98, 1.59)	0.07
eGFR <60 mL/min per 1.73 m^2($n = 414$)					
n with plaque	30	60	39	36	
n without plaque	68	78	60	43	
OR (95% CI)	1.0	1.79 (1.00, 3.19)	1.53 (0.83, 2.84)	2.16 (1.10, 4.24)	0.06

Note: Model 3 from Table 2: Adjusted for sex, age, waist circumference, total cholesterol/HDL ratio, C-reactive protein, statin treatment, HbA1c, education level, physical activity, and when appropriate also for smoking and hypertension. Complete case analyses based on $n = 3{,}807$ (men and women), except for the model stratified for menopausal status ($n = 2{,}298$ women with complete data for all covariates and menopausal status) and for eGFR categories ($n = 3{,}777$ men and women with complete data for all covariates and eGFR). CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; OR, odds ratio; Q, quartile.

[a]Linear trend *p*-values were derived by modeling the median value of each blood lead quartile as a continuous variable in the models.

[b]$p_{Interaction}$ term of Q4 × ever smokers in Model 3 with all individuals = 0.10.

[c]$p_{Interaction}$ term of Q4 × hypertension in Model 3 with all individuals = 0.82.

[d]$p_{Interaction}$ term of Q4 × postmenopausal status in Model 3 with all individuals = 0.11.

[e]$p_{Interaction}$ term of Q4 × eGFR <60 mL/min per 1.73 m^2 in Model 3 with all individuals = 0.48.

estimates (see Tables S7 and S8). Adjusting Model 3 for BMI instead of waist circumference showed essentially the same results (see Table S9).

When we stratified Model 3 by age (< and ≥55 y), associations were stronger among individuals >55 years of age as compared with those <55 years of age among both men and women together [OR for Q4 vs. Q1 = 1.45 (95% CI: 1.13, 1.85) in those >55 years of age, and OR for Q4 vs. Q1 = 1.08 (95% CI: 0.72, 1.85) in those <55 years of age] as well as among men [OR = 1.23 (95% CI: 0.81, 1.88) and OR = 1.04 (95% CI: 0.52, 2.07), respectively] and women [OR = 1.74 (95% CI: 1.26, 2.40) and OR = 1.10 (95% CI: 0.63, 1.94), respectively], separately (see Table S10).

When we ran the analyses with B-Pb as a continuous variable rather than in quartiles, every 10-μg/L increase in B-Pb was associated with a 12% higher risk (95% CI: 1.05, 1.19) of atherosclerotic plaque in women and 5% higher risk in both men and women together (95% CI: 0.99, 1.10), whereas there was no association among men [OR = 0.98 (95% CI: 0.91, 1.05)]. When we stratified by postmenopausal status, every 10-μg/L increase in B-Pb was associated with an 18% higher risk (95% CI: 1.09, 1.27) among postmenopausal women, whereas there was no association among premenopausal women [OR = 0.95 (95% CI: 0.78, 1.13)].

Finally, we excluded all individuals with B-Pb above 100 μg/L ($n = 8$ men and $n = 7$ women) to test whether the associations found between B-Pb (as a continuous variable) and risk of atherosclerosis were driven by individuals with extreme values. The results were essentially the same for all individuals [OR = 1.06 (95% CI: 1.002, 1.12)] as well as for women [OR = 1.12

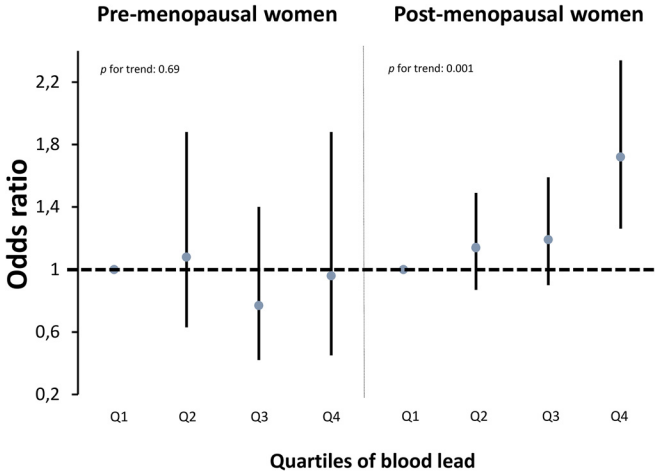

Figure 2. Odds ratios for atherosclerotic plaque associated with blood lead concentrations (quartiles Q1–Q4) in women by menopausal status ($n = 603$ premenopause, $n = 1{,}695$ postmenopause), adjusted for smoking, waist circumference, total cholesterol/HDL ratio, hypertension, age, CRP, statin treatment, HbA1c, education level, and physical activity. Vertical lines indicate 95% confidence intervals. $p_{Interaction}$ for Q4 vs. Q1 was 0.11. Note: CRP, C-reactive protein; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein.

(95% CI: 1.04, 1.21)] and men [OR = 1.001 (95% CI: 0.92, 1.08)] separately (see Table S11).

Discussion

Our study, based on a large Swedish population-based cohort, supports the hypothesis that environmental lead exposure is an important risk factor for atherosclerosis. After controlling for known risk factors and confounders, individuals in the highest quartile of B-Pb (median: 42 μg/L; range: 33–258) had a 35% (95% CI: 9, 66%) higher risk of atherosclerosis compared with those in the lowest quartile (median: 15 μg/L; range: 1.5–18). In men, there was a nonsignificant 18% (95% CI: –17, 69%) increase in OR, whereas the associations were stronger for women, who showed a 58% (95% CI: 20, 108%) increased risk in the highest quartile of B-Pb compared with the lowest quartile. However, among women, the association between B-Pb and plaque was limited to postmenopausal women; OR for Q4 vs. Q1 = 1.72 (95% CI: 1.26, 2.34) vs. OR for Q4 vs. Q1 = 0.96 (95% CI: 0.49, 1.89) in premenopausal women. Individuals with decreased kidney function (eGFR <60 mL/min per 1.73 m^2, $n = 414$) had a higher risk of atherosclerosis as compared with those with normal kidney function.

As discussed in several reviews and reports (EFSA 2010; Navas-Acien et al. 2007; NTP 2012; Solenkova et al. 2014), a number of studies have shown associations between environmental lead exposure and cardiovascular disease and mortality in cohorts with low-level lead exposure (B-Pb concentrations <50 μg/L). Five American studies on low-level environmental lead exposure (NHANES data) showed consistent associations between B-Pb concentrations and cardiovascular mortality (Lanphear et al. 2018; Lustberg and Silbergeld 2002; Menke et al. 2006; Ruiz-Hernandez et al. 2017; Schober et al. 2006). An increased risk of cardiovascular mortality has also been found at high levels of lead exposure in several occupational cohorts (Navas-Acien et al. 2007; Skerfving and Bergdahl 2015). Concerning nonfatal clinical cardiovascular outcomes, three American studies based on NHANES cohorts showed an association between low-level lead exposure and peripheral arterial disease (Guallar et al. 2006; Muntner et al. 2005; Navas-Acien et al. 2004), whereas another study using lead concentrations in urine (a less reliable biomarker of lead exposure) showed no associations (Navas-Acien et al. 2005). The systematic review by Chowdhury et al. (2018) identified only two prospective studies on environmental lead exposure and coronary artery disease or stroke: one British and one Danish (Møller and Kristensen 1992; Pocock et al. 1988). These prospective studies found little support of associations between B-Pb concentrations and coronary heart disease after adjustment for confounders (Møller and Kristensen 1992; Pocock et al. 1988). In contrast, an American study based on the Normative Aging Study cohort reported an increased incidence of coronary heart disease [Hazard ratio = 1.73 (95% CI: 1.05, 2.87)] among individuals with B-Pb ≥50 μg/L vs. <50 μg/L, even after adjustment for risk factors and confounders (Jain et al. 2007). Finally, a study in Chinese adults reported no significant trend between plasma lead levels and incident coronary heart disease using plasma lead modeled by quartiles (Yuan et al. 2017), but plasma lead is rarely used as a biomarker for lead exposure. The level of lead in plasma is usually <1% of that in whole blood and the plasma levels in the study by Yuan et al. (2017) were much higher than what can be expected in a population study (Hernández-Avila et al. 1998; Skerfving and Bergdahl 2015).

Cardiovascular disease continues to be the leading cause of death and the main contributor to premature mortality worldwide (GBD 2016 Causes of Death Collaborators 2017). In particular, coronary heart disease and cerebrovascular disease together account for about 15 million deaths globally every year (GBD 2016 Causes of Death Collaborators 2017). Lead exposure has been estimated to account for 2% of the total cardiovascular disease burden worldwide (nearly 240,000 deaths yearly), suggesting lead to be an important cardiovascular risk factor (GBD 2015 Causes of Death Collaborators 2016). Taking into account that lead exposure is widespread and that 41% of all individuals in the highest quartile of B-Pb had atherosclerotic plaque in the carotid artery, the results in the present study are relevant for public health.

In our study, the association was limited to postmenopausal women with those in the highest quartile of B-Pb showing a 72% higher risk of plaque (95% CI: 1.26, 2.34) in comparison with those in the lowest quartile. In premenopausal women the corresponding OR was 0.96 (95% CI: 0.49, 1.89). However, when we examined the associations between B-Pb and plaque among men and women by age (< and ≥55 y), estimates were somewhat stronger also among men in the older group as compared with the younger group, although nonsignificant for men. Therefore, we cannot rule out that the associations found among postmenopausal women are partially driven by age. Few studies have examined lead-related cardiovascular risks in postmenopausal women. Nash et al. (2003) found an increasing risk of diastolic hypertension with increasing quartiles of B-Pb in postmenopausal women, whereas another small study found no associations (Al-Saleh et al. 2005). Menopause is known to involve increased bone demineralization, meaning that lead stored in bone may enter the blood stream at a higher rate and thus increase the circulating lead concentrations and thereby the endogenous lead exposure (Silbergeld et al. 1988; Symanski and Hertz-Picciotto 1995). However, the postmenopausal women in our study showed only slightly higher B-Pb concentrations (median 23 μg/L) compared with premenopausal women (21 μg/L; $p < 0.001$). Thus, if our finding regarding effect modification by menopause is valid (the numbers of premenopausal women per quartile were limited), it may be due to other factors that are more prevalent throughout this period of life, rather than solely a higher endogenous lead exposure. Both vascular endothelial dysfunction (VED) and large artery stiffness are known to accelerate during menopause, presumably as consequences of a declining ovarian function and

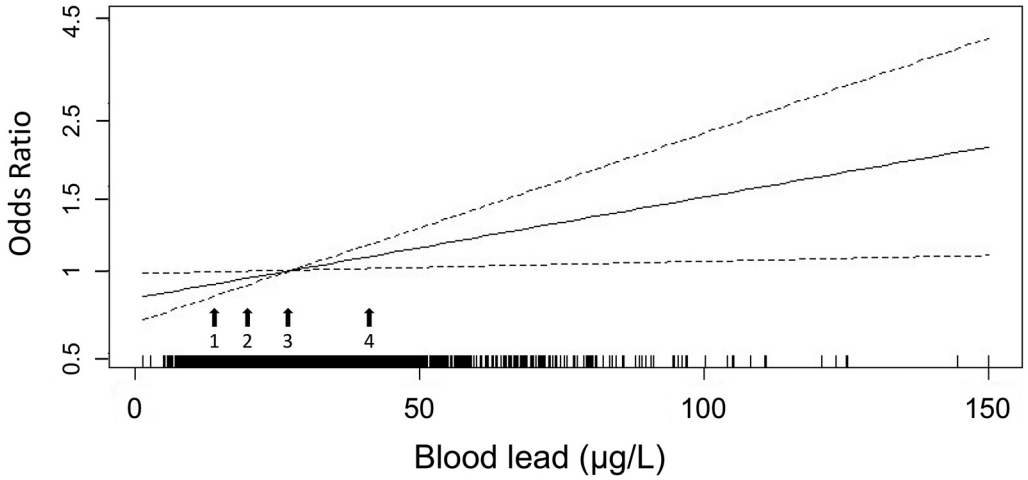

Figure 3. Smooth function (with 95% confidence interval) describing the impact of blood lead (B-Pb) (below 150 µg/L, $n = 4{,}170$) on the risk of atherosclerosis. Model adjusted for smoking, waist circumference, total cholesterol/HDL ratio, hypertension, age, sex, CRP, statin treatment, HbA1c, education level and physical activity (Model 3 from Table 2). Individual values of B-Pb are indicated on the *x*-axis and arrows indicate medians of B-Pb within quartiles. Log (odds) relabeled to odds ratios are presented. Note: CRP, C-reactive protein; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein.

decreased estrogen levels (Moreau and Hildreth 2014). The faster progression of VED during menopause has been associated with a decreased availability of nitric oxide as well as an up-regulation of pro-inflammatory cytokines: two mechanisms that have been associated with lead exposure (Vaziri 2008).

Several mechanisms have been suggested to underlie lead-induced cardiotoxicity (Vaziri 2008). Cellular and molecular mechanisms such as oxidative stress, increased lipid peroxidation, impairment of the renin–angiotensin system, endothelial injury with reduced endothelial cell growth, and stimulation of the vascular smooth muscle cell proliferation have been shown experimentally. Interestingly, low-level lead exposure has been associated with increased pulse pressure, suggesting the involvement of lead in the pathogenesis of arterial stiffness (Perlstein et al. 2007). Large artery stiffness has been attributed to structural alterations in the arterial wall such as increased collagen and decreased elastin but also to functional alterations related to a decreased nitric oxide availability (Moreau and Hildreth 2014). In turn, increased artery stiffness has been associated with a higher prevalence of atherosclerosis, independent of other cardiovascular risk factors (Zureik et al. 2002). An *in vitro* study showed that lead levels of 0.5–10 µM induced proliferation of vascular smooth cells and fibroblasts (Fujiwara et al. 1995). In another study, Zeller et al. (2010) found that lead was absorbed by human endothelial cells (ECs) and smooth muscle cells (SMCs). Lead caused proliferation of ECs and up-regulation of cellular stress proteins (heme oxygenase-1, heat shock protein 7, and 4-nitroquinoline 1-oxide) and increased secretion of IL-8. This stimulated the invasion of SMCs into the intima and intima thickening, as demonstrated in a human arterial organ culture. In addition, lead stimulated elastin synthesis in SMCs, which is of interest because elastic fibers are primary sites for lipid deposition. These findings suggest mechanisms whereby lead may be involved in the initiation phase of atherosclerosis (Zeller et al. 2010).

In humans, evidence on low-level lead toxicity in the cardiovascular system is particularly extensive for hypertension, which is an important risk factor for cardiovascular disease (Navas-Acien et al. 2007; NTP 2012; U.S. EPA 2006). However, limited data are available on associations between lead exposure in the general population and atherosclerosis, a key mechanism in cardiovascular disease. We found three studies investigating the relation between B-Pb concentrations and ankle–brachial index (ABI), an indirect measure of atherosclerosis in the lower extremities (Guallar et al. 2006; Muntner et al. 2005; Navas-Acien et al. 2004). In all three studies, B-Pb concentrations were associated with peripheral artery disease (ABI <0.9). Although there is some evidence from experimental studies linking lead exposure to atherosclerosis, to our knowledge, only one epidemiological study has investigated the association between environmental lead exposure and atherosclerotic plaques in the carotid arteries (Lind et al. 2012). The latter study was performed in 1,016 elderly individuals and found no significant association with B-Pb. The present epidemiological study is the first to show an association between B-Pb and the risk of atherosclerosis in the carotid arteries.

Individuals with impaired renal function might be a susceptible population at risk of cardiovascular effects of lead. Reduced kidney function could lead to a less efficient elimination of lead due to a reduced renal clearance, with increased B-Pb concentrations as a consequence (NTP 2012). This could in turn increase the risk of developing atherosclerosis earlier. In the present study, however, blood concentrations in individuals with decreased kidney function defined as eGFR <60 mL/min per 1.73 m^2 (median B-Pb: 23.5; range: 6.1–173) did not differ statistically from those in individuals with normal kidney function (median: 24.2; range: 1.5–258; $p = 0.20$). Moreover, adjusting the statistical models for eGFR did not affect the estimates. Nonetheless, reduced kidney function is well known to be associated with higher cardiovascular risk (Anavekar et al. 2004; Go et al. 2004). In our study, individuals in the highest quartile of B-Pb with eGFR <60 mL/min per 1.73 m^2 had a higher risk of developing atherosclerosis [OR = 2.16 (95% CI: 1.10, 4.24)] compared with those with normal kidney function, but this was based on few individuals and results should, therefore, be interpreted with caution. However, the relation between lead exposure and kidney function is complex. Apart from the potential impact of GFR on B-Pb levels, the reverse may also be the case, that is, that lead exposure may cause kidney damage. This has been shown also in prospective studies, including a recent one based on the present cohort (Harari et al. 2018). Here we used serum creatinine to estimate GFR. Combined creatinine/cystatin C equations may be superior (Spector et al. 2011), but in the present cohort the impact of B-Pb on the longitudinal change of eGFR was similar for the creatinine equation and the combined equation (Harari et al. 2018).

Environmental exposure to lead remains a public health problem. Although lead exposure has decreased considerably in recent years (Skerfving and Bergdahl 2015), it still occurs via contaminated food and drinking water, smoking, and inhalation of polluted

air in areas with heavy traffic or industrial emissions (EFSA 2010; Skerfving and Bergdahl 2015). As a consequence, there are still reports of health effects in children and adults even at low levels of lead exposure (NTP 2012; Skerfving and Bergdahl 2015). B-Pb concentrations in the present study ranged from 1.5 to 258 μg/L (median: 25), which is in line with those reported in other studies on environmental lead exposure (Åkesson et al. 2005; Buser et al. 2016; de Burbure et al. 2003; Sommar et al. 2013; Spector et al. 2011; Yu et al. 2004), but much lower than in occupational settings (Navas-Acien et al. 2007; Skerfving and Bergdahl 2015). The associations between B-Pb and risk of atherosclerosis in our study were found in individuals in the highest quartile of B-Pb (median: 42 μg/L; range: 33–258), which is higher than the levels at which associations with peripheral artery disease were found in the NHANES studies (Guallar et al. 2006; Muntner et al. 2005; Navas-Acien et al. 2004). The associations remained essentially the same after excluding individuals with B-Pb >100 μg/L.

The present study has several strengths and limitations. Strengths include the large number of participants (although the numbers were small in some stratified analyses), the high analytical accuracy of the B-Pb analyses, and the standardized ultrasound technique. Another strength was the possibility to adjust the statistical models for the main known cardiovascular risk factors and potential confounders. This study also has limitations. For example, due to the cross-sectional study design, we cannot infer the temporal relation between exposure and the outcome. In addition, we used a single measurement of B-Pb as a measure of lead exposure, and it is likely that B-Pb for many of the participants had been higher before the early 1990s when they were recruited (Elinder et al. 1986). Therefore, we cannot exclude the possibility that development of atherosclerosis in the study participants was at least partly due to higher lead exposure earlier in life. Bone lead, which was not available, would have been a better biomarker of long-term cumulative lead exposure. B-Pb has a half-life of about 1 month after end of exposure, so if exposure suddenly increases or decreases, this will affect B-Pb levels. At a stable exposure it will, however, reflect the body burden. We did not have complete data on pack-years for all individuals, and therefore used smoking status (never or ever smoker) instead. Nevertheless, sensitivity analyses adjusted for pack-years showed essentially the same results as those adjusted for smoking status. We did not include dietary factors among potential confounders adjusted for, but a proxy of overall energy intake should be covered by the inclusion of waist circumference. Regarding fiber, fish, fruit and vegetables, we do not consider them related to lead intake. Another limitation is the use of only the presence of plaque as outcome. Other measurements such as the plaque size or plaque burden would have been useful measurements of the degree of atherosclerosis. Such measurements were, however, not done in a consistent way in the early 1990s when the study recruitment was performed.

In conclusion, our study shows an association between lead exposure and the occurrence of atherosclerotic plaque in the carotid artery, adding evidence to an underlying pro-atherogenic role of lead in cardiovascular disease.

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